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# **Differentiation in Microgravity and in Analog Culture**

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# Differentiation

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**The act or process of acquiring completely individual characteristics as occurs in the progressive diversification of cells and tissues during embryogenesis.**



# Differentiation in Microgravity and in Analog Culture

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- Cells in continuous fall culture exhibit several characteristics of adaptation
- The least understood is the propensity for differentiation
- Likewise there is a similar observation for cells on microgravity
- The microgravity experience is far less than the analog culture systems in 1 G
- There is an emerging hypothesis that the observed differentiation follows the physical changes in morphology and lowers the threshold for induction of differentiation
- The differentiation may be a combinatorial response to altered signal transduction, cytoskeletal organization, and membrane fluidity
- In summary, *'the jury is out'*



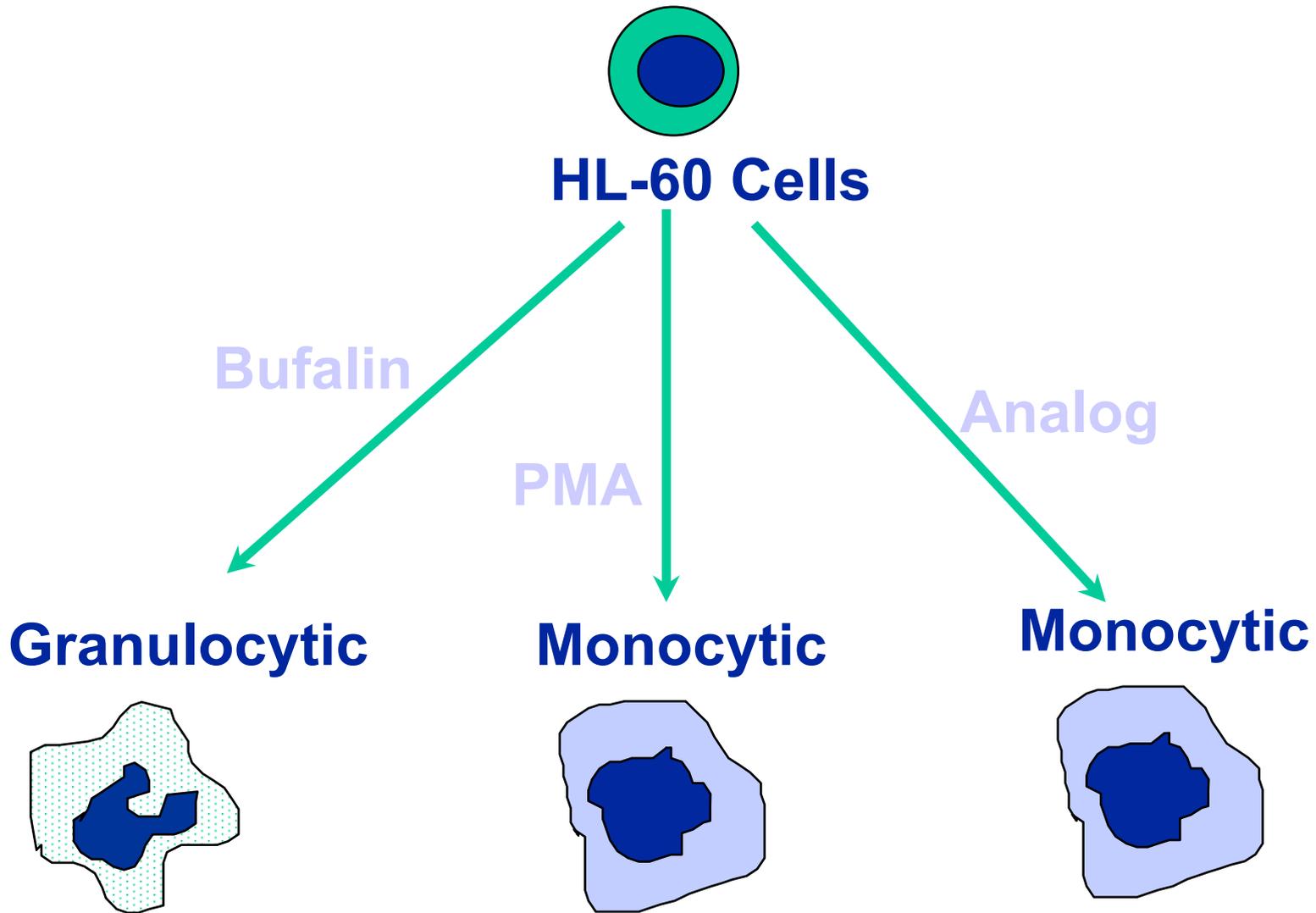
# The Question of Differentiation

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- **Model Requirements**
  - Free living cell system
  - Morphological markers
  - Cell surface and molecular genetic markers
  - Amenable to analysis on the RWV
- **Model Cell Systems**
  - Stem Cell Leukemias
  - HL60 Human promyelocytic leukemia



# HL-60 Promyelocytic Differentiation Model





# Differentiation in the Analog Culture System

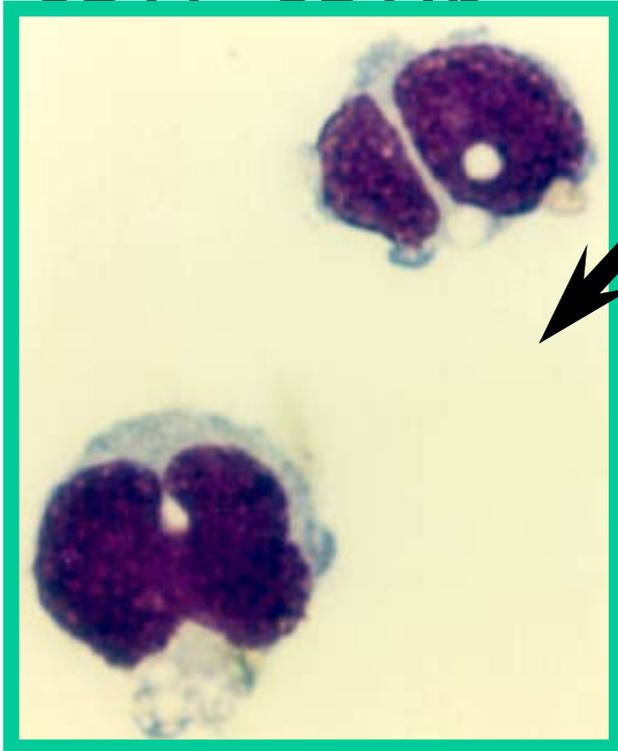
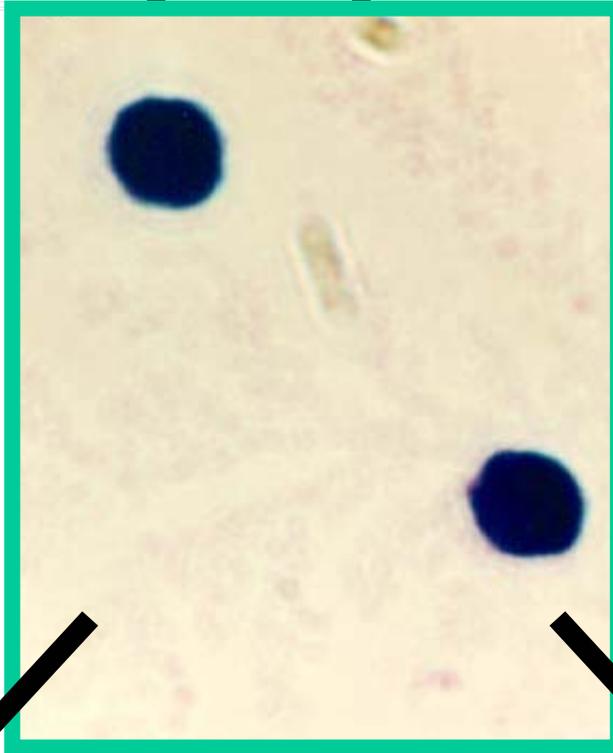
## HL-60 Promyelocytic Leukemia

RWV

Static

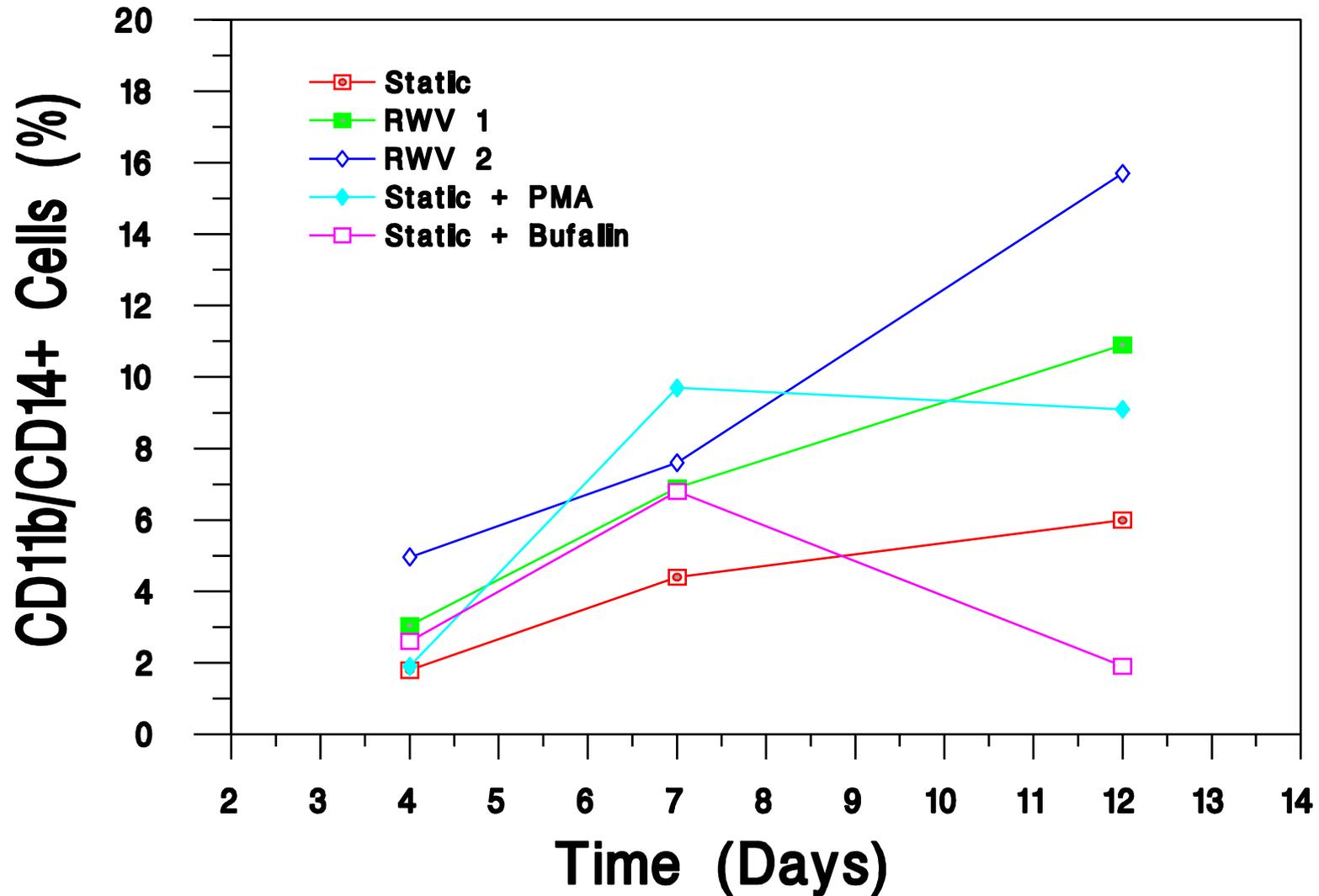
Monocyte  
CD14+ CD11b +

CD14- CD11b -



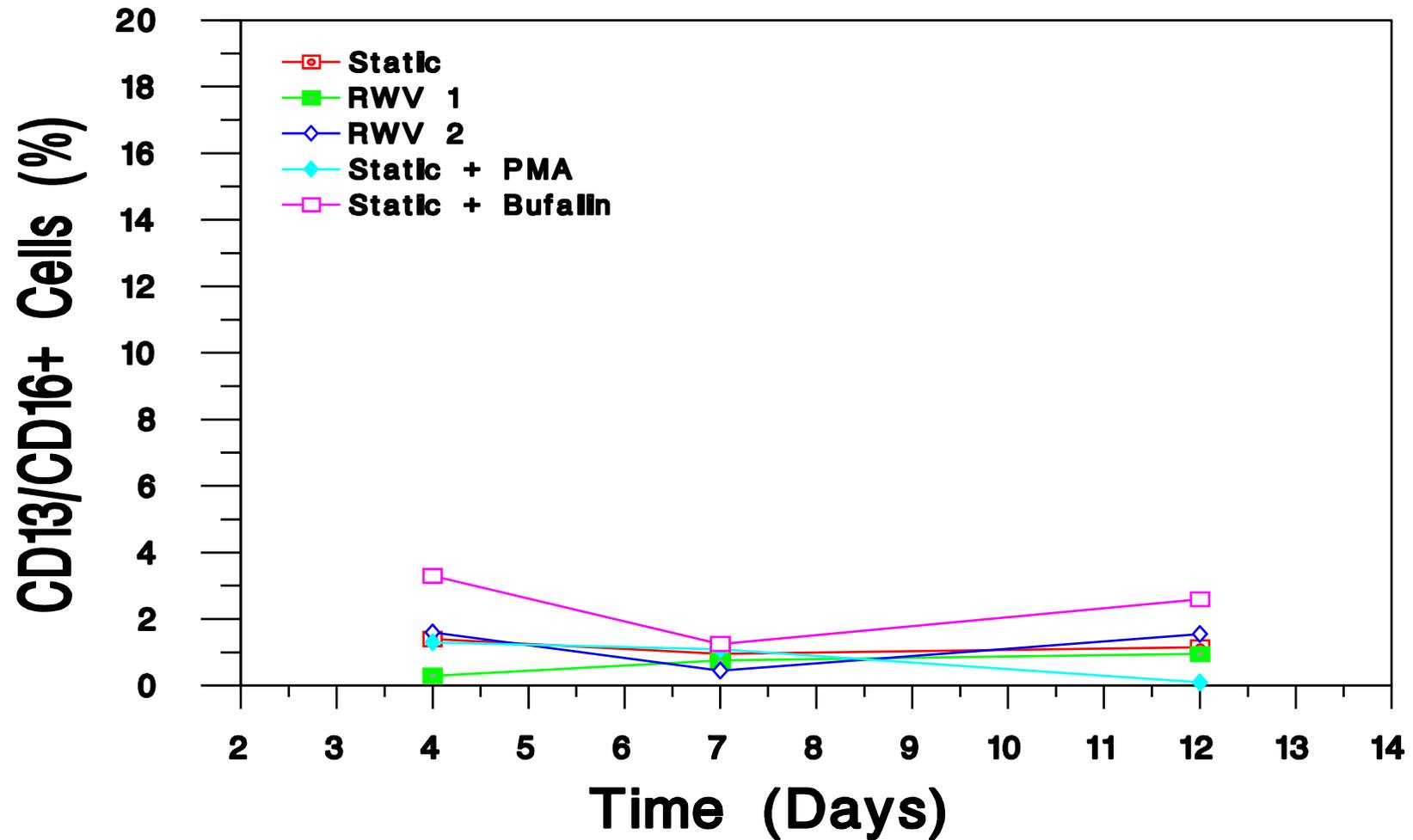


# Monocyte Differentiation in the NASA Bioreactor





# Granulocyte Differentiation in the NASA Bioreactor





# HL-60

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- **CD-14/CD-11b coexpression**
- **Upregulation of p53-like gene**
- **Upregulation of alcohol dehydrogenase**
- **Monocyte morphology**



# **Microgravity and Gene Expression**

## ***Preliminary Results:***

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- 1. In simulated microgravity leukemic stem cells differentiate and change gene expression.**
- 2. Using a limited set of primers and RT-PCR we observed at least six potential candidate genes that are expressed in rotational culture.**
- 3. Two have been amplified, cloned, and sequenced. One is 70% homologous with p53 and the other with alcohol dehydrogenase.**
- 4. In human renal cells approximately 40 genes evidence changed expression in free fall, while over 800 from panel of 10,000 are changed.**
- 5. None of these are the expected stress response suite observed in heated or sheared cells.**



# RT-PCR Runout of HL-60 Cells In the Bioreactor

Bioreactor



1000 base pairs



# **Microgravity and Gene Expression**

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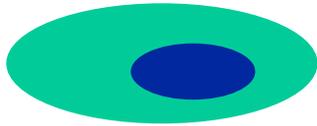
## **Significance**

- 1. Absence of gravity constitutes a physical manipulation that alters gene expression.**
- 2. Possibly novel genes may be found as seen in the heat shock proteins.**
- 3. May provide insight into the adaptational response of humans to microgravity.**
- 4. May be a consequence of altered signal transduction.**

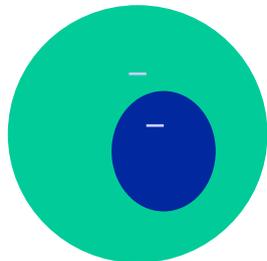


# Model for Microgravity Induced Differentiation

1g



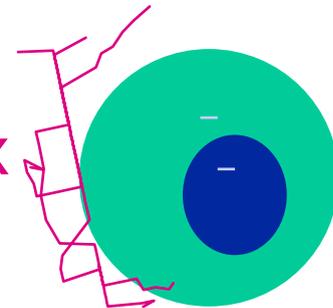
$\mu$ g



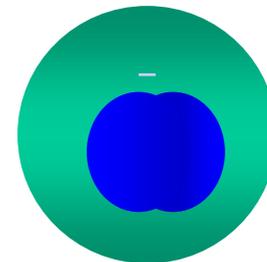
p53-like moiety



p21



Stem cell



CD11b+  
CD14+  
Monocyte

## Adaptational Responses

Shape change

Cytoskeletal array

Signal transduction loss

Gene expression

Gene products

Loss of polarization and movement



# Conclusions

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- **HL60 is a useful model in assessing the role of randomized gravity in differentiation**
- **RWV culture may favor selected differentiation**
- **This may explain the tissue diversification observed in cocultures**



# Cartilage

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- **Alkaline phosphatase production**
- **Synthesis of Type 2 collagen**
- **Glycosamino glycan production**
  - **Chondroitin 4 SO<sub>4</sub>**
  - **Chondroitin 6 SO<sub>4</sub>**
- **Lacunae**
- **Chondrocyte Morphology**

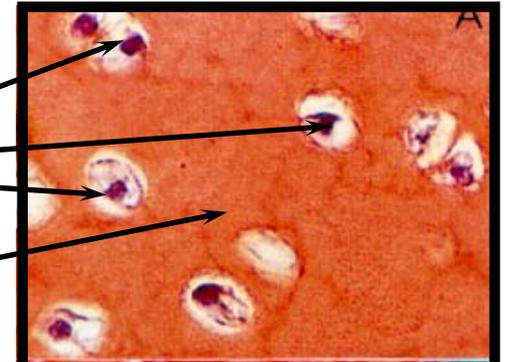


# Cartilage Development in the NASA Bioreactor

**Native cartilage**

Lacunae

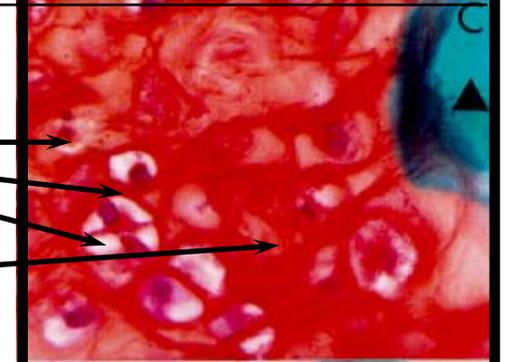
Matrix



**Bioreactor**

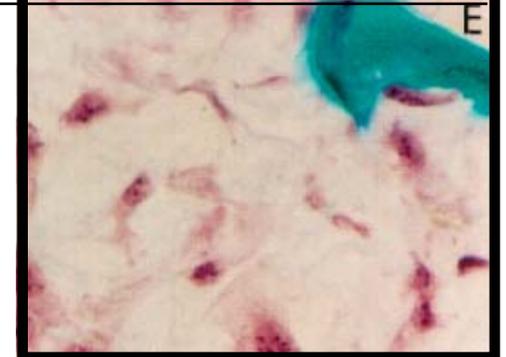
Lacunae

Matrix



**Stationary Culture**

No Lacunae  
No Matrix





# Renal Proximal Tubule Cells

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- **Microvilli**
- **Secretion of erythropoietin**
- **1, 25 dihydroxy-vitamin D3**
- **Megalin**
- **Cubulin**
- **Gene expression changes**



# Renal Proximal Tubular Cells

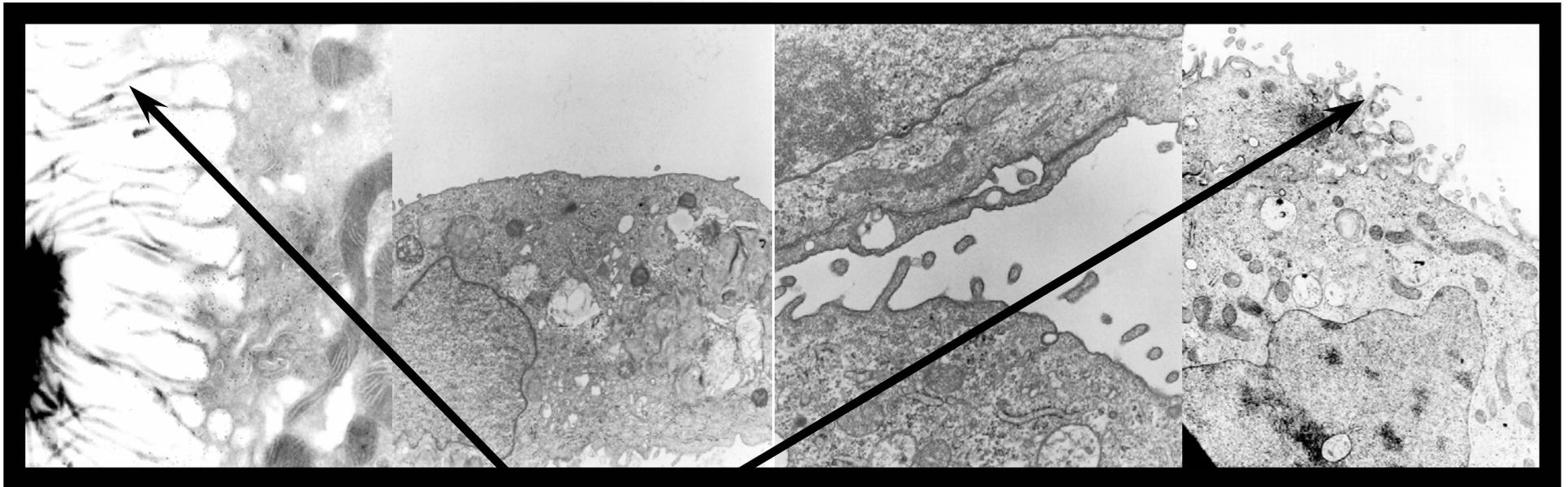
Dr. Timothy Hammond, Tulane Univ. Med.Ctr.  
(An in vitro test for renal toxicity)

Native  
Specimen

Standard  
Culture

STLV

STLV  
Co-culture



Microvilli with receptors for toxic substances  
Production of EPO



# Pheochromocytoma

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- **Adrenal medullary tumor**
- **Paraganglion origin**
- **Can cause severe catecholamine induced hypertension**
- **Chromaffin granules**
- **May produce steroid like hormones**

**Pheochromocytoma**  
**PC-12**

- **Dopamine- $\beta$ -hydroxylase**
- **Pheny-n-methyltransferase**



# Colon carcinoma

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- **Gland formation**
  - CEA production
  - Mucin
- **Epidermal growth factor**
- **Epidermal growth factor receptor**
- **Transforming Growth Factor  $\beta$**
- **Altered cyclin expression**



# Colon carcinoma

**Stationary  
Culture**

Monolayer

**Bioreactor  
3-D Culture**

Polypoid  
Structure

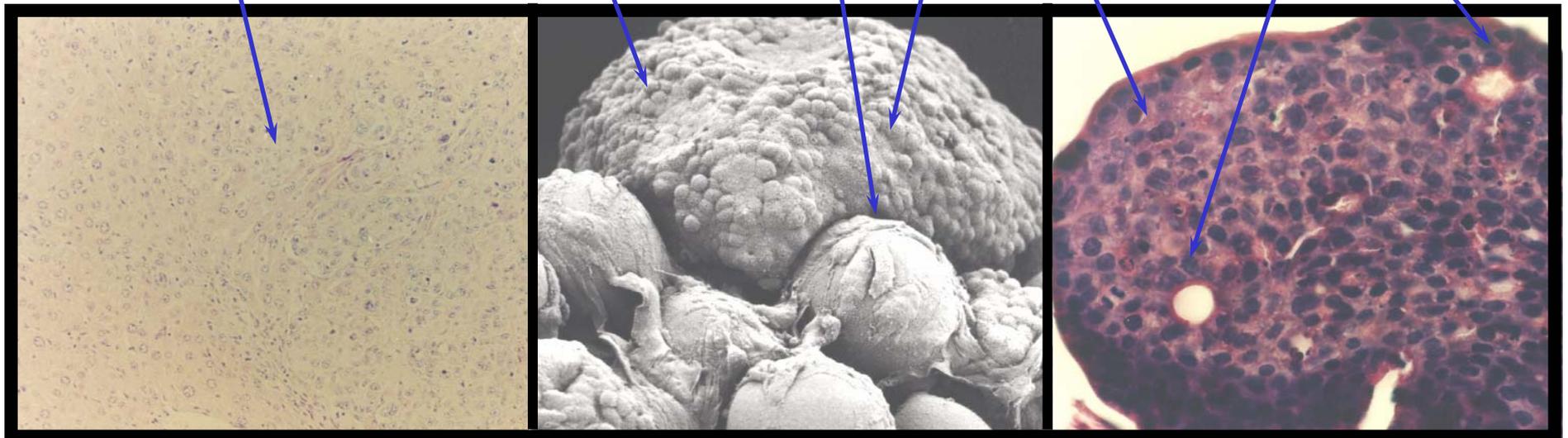
Fibroblasts

Neoplastic Cells

**Bioreactor  
3-D Culture**

**Histology**

Gland-like  
Structures





# Breast carcinoma

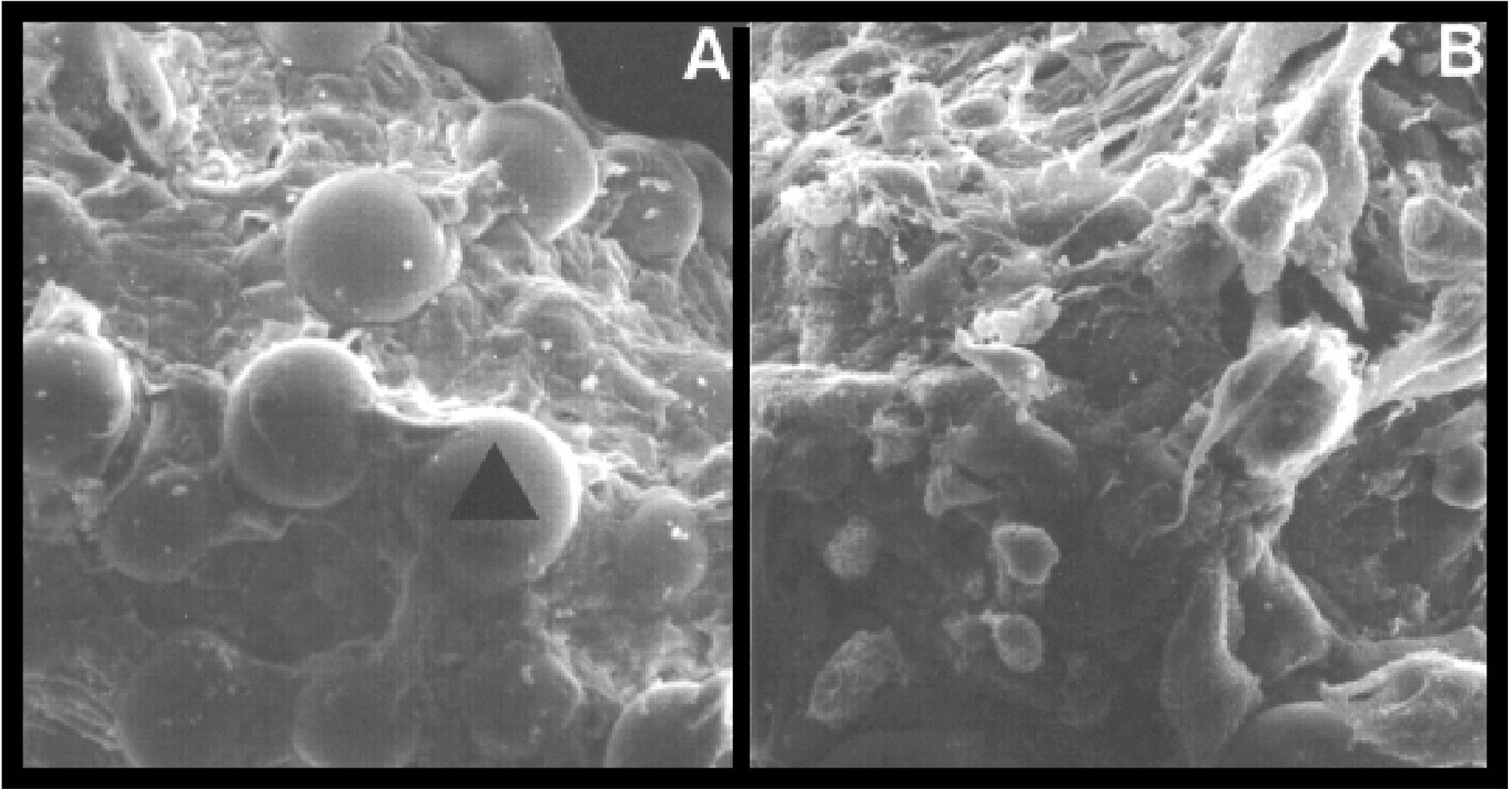
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- **Estrogen receptor**
- **p53**
- **p21**
- **vimentin**
- **c-erb-2**
- **propagation of primary biopsy specimens**



# Primary Breast Carcinoma

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*Becker*



# Endothelial cells

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- **PECAM-1**
- **von Willebrand's Factor**
- **Factor 8**

**Further differentiation of the endothelial cells may require re-introduction of hydrodynamic shear**

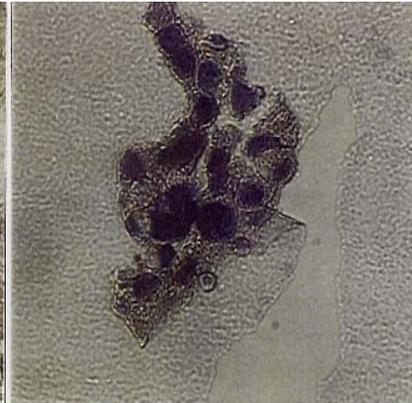


# Endothelial cells

## Expression of von Willebrand's Factor

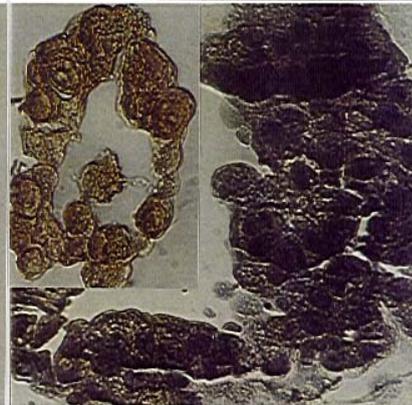
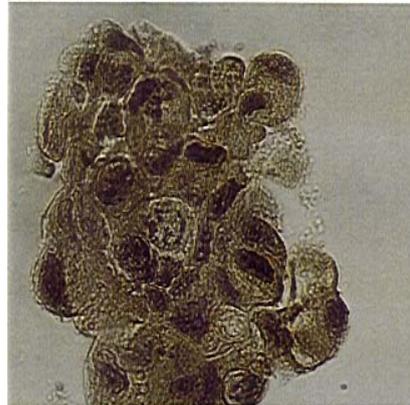
**Microgravity**

**14 days**

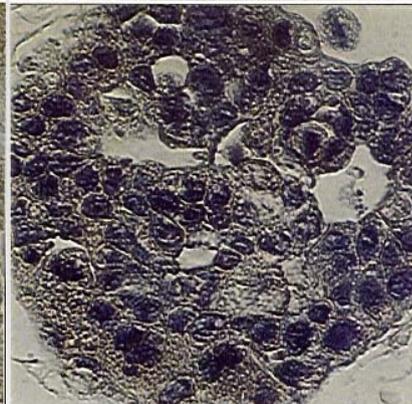


**Unit gravity**

**28 days**



**42 days**





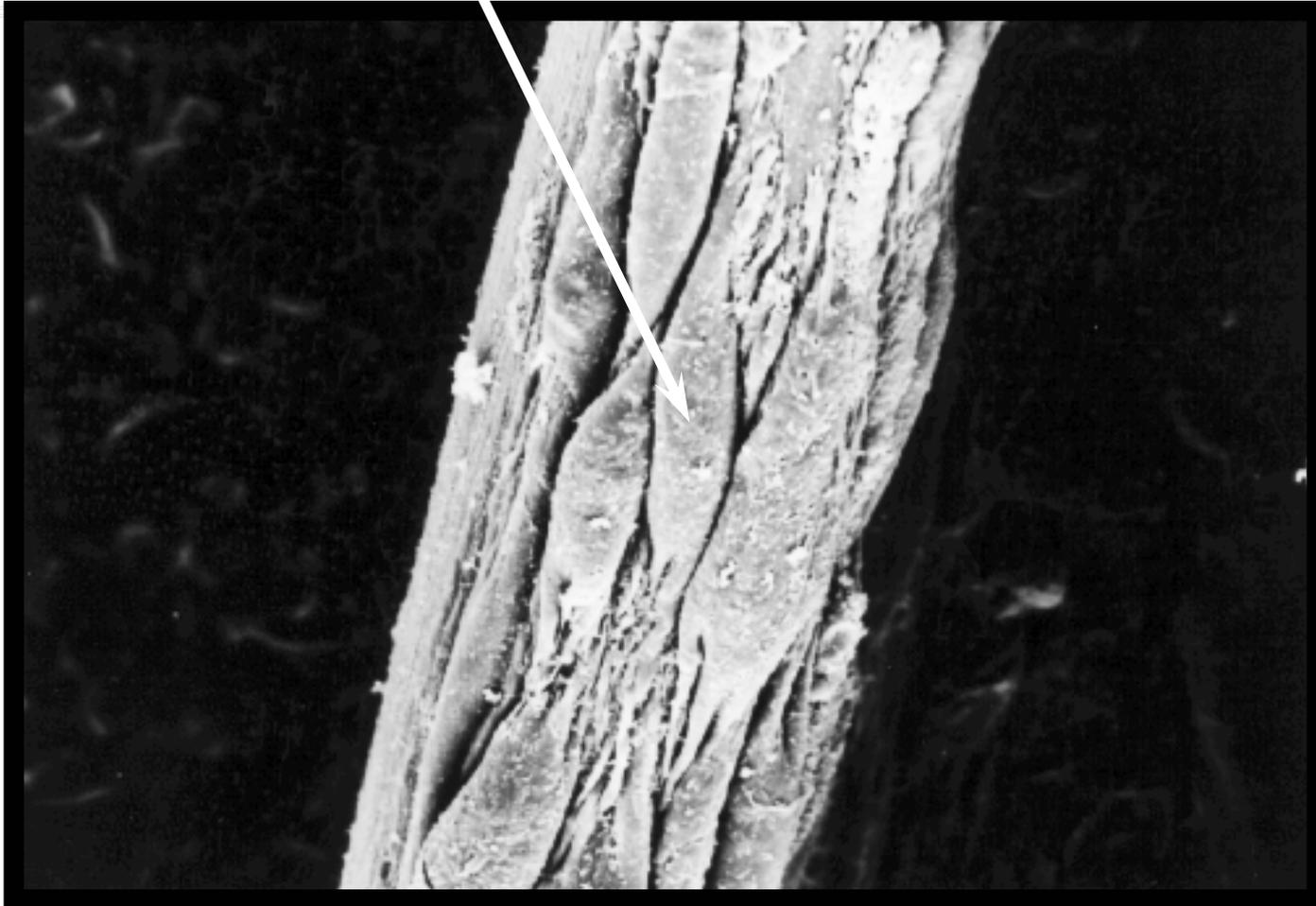
# Cardiac myocytes

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- **Linearized assembly with coordinated contraction**
- **Switch from  $\beta$  to  $\alpha$  myosin**
- **Response to cardioregulatory drugs**



## Heart muscle cells aligning and growing on connective tissue fiber



Control: 40-45 bpm

Isoprenalol: 60-65 bpm

*Drs. Hartzell and Akins*

*Dupont*

Tissue  
Engineering



# Ovarian Cancer

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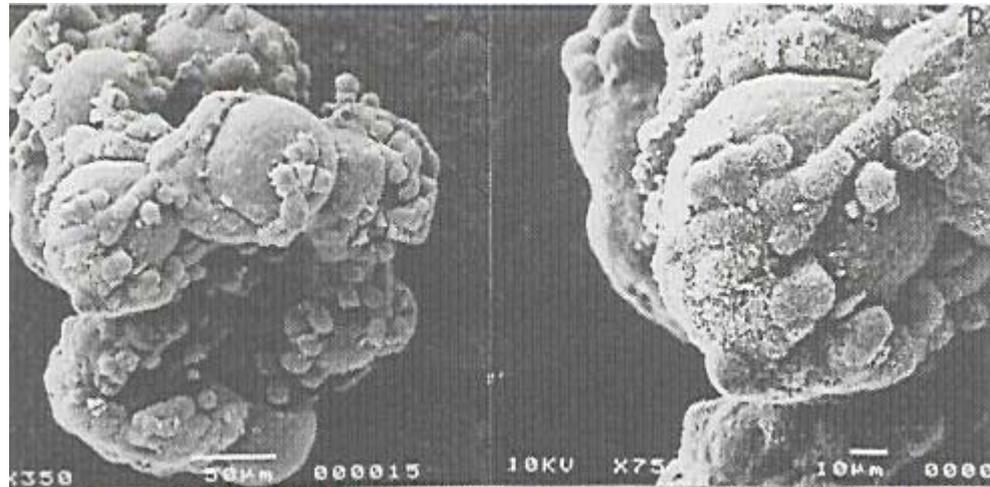
- **Mixed muellerian type**
- **p53**
- **p21**
- **TAG72**
- **c-erb-2**



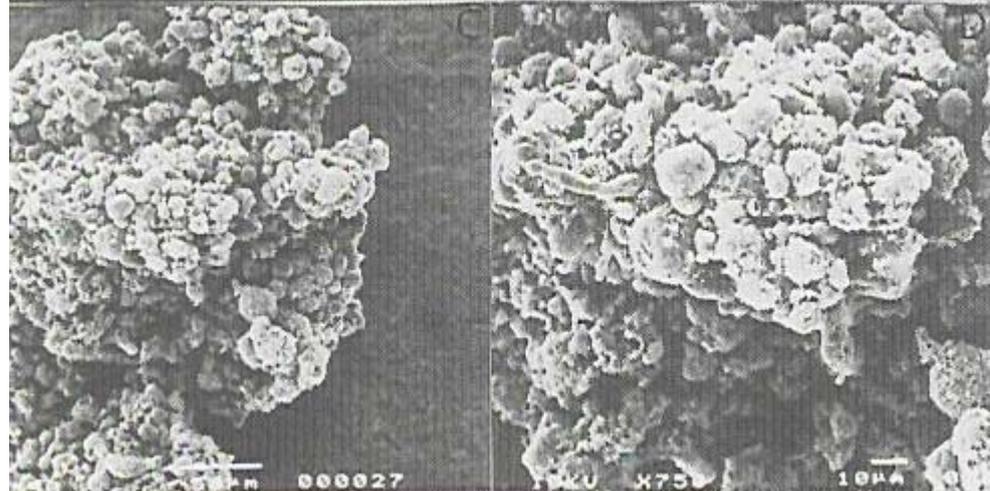
# Ovarian Carcinoma

## *Analog Culture*

**4 days**



**32 days**

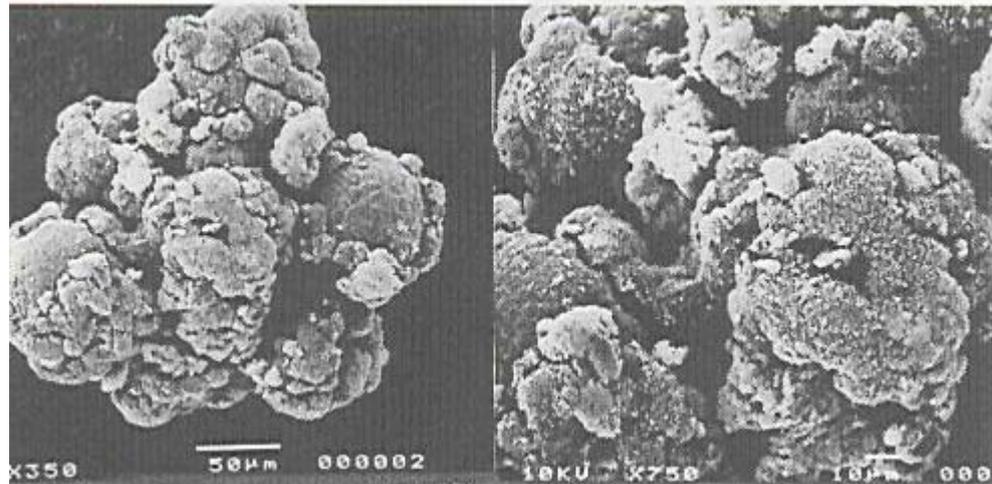




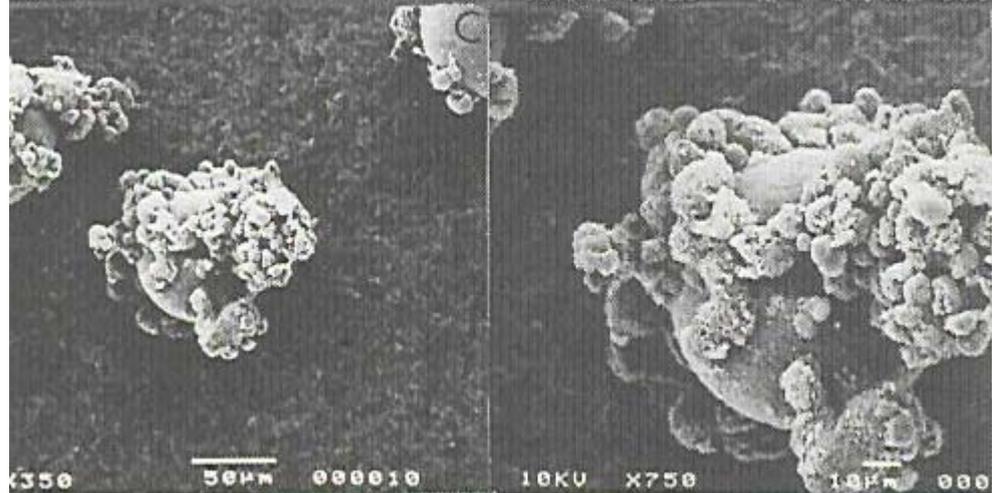
# Ovarian Carcinoma

## *Petri Dish Culture*

**4 days**

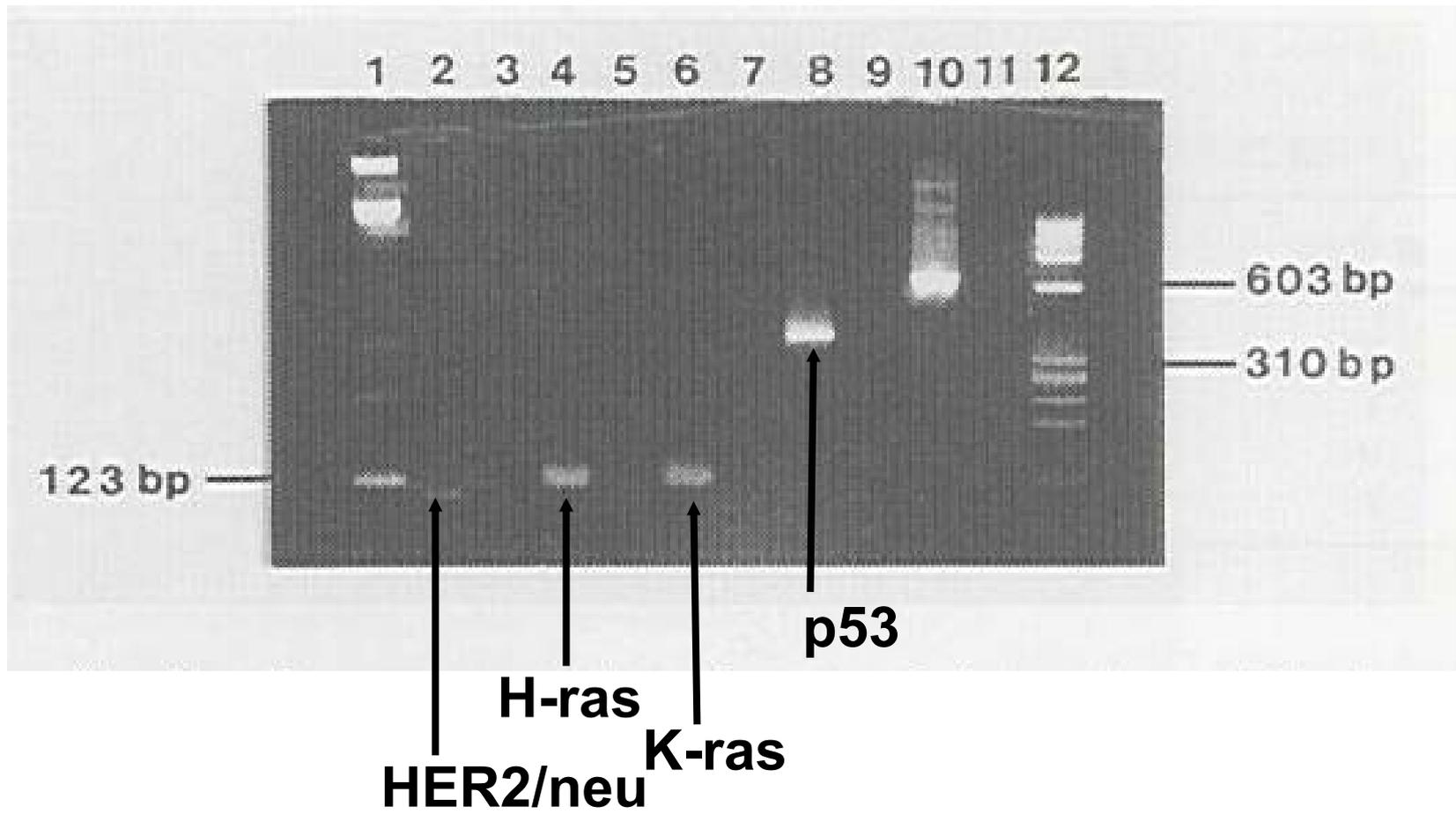


**32 days**





# Ovarian Carcinoma





# Prostate cancer

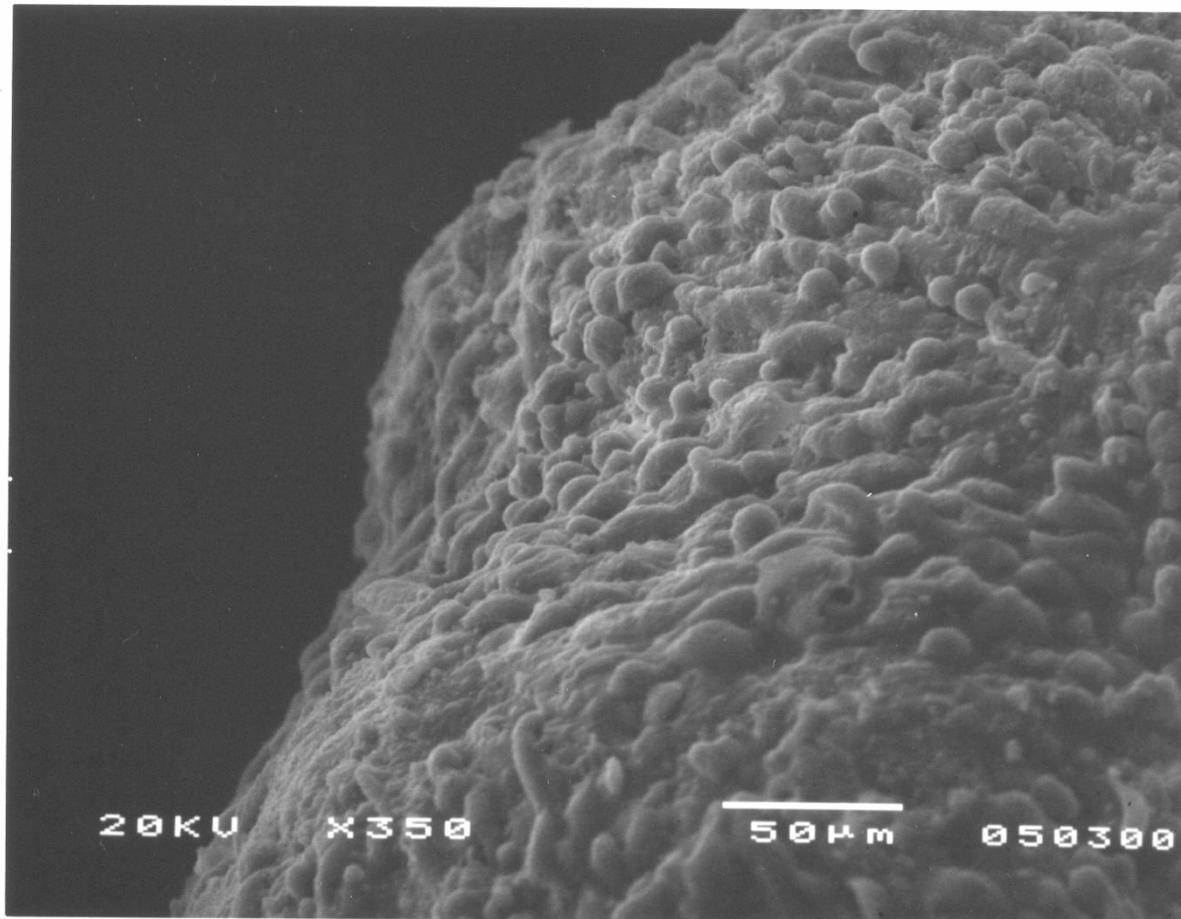
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- **Prostate specific antigen**
- **Epidermal growth factor**
- **acid FGF**
- **Vascular endothelial growth factor (VEGF)**



# Prostate cancer

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# Summary

- **Propagation of Cells for Tissues**

- Cancer models
  - Colon
  - Breast
  - Ovarian
  - Prostate
  - Endocrine
- Normal Tissue
  - Cartilage
  - Liver for hepatitis pathogenicity model
  - Human Kidney
  - Liver for extracorporeal support
  - Lymphoid tissue for HIV pathogenesis
  - Thyroid
  - Skin
  - Pancreatic islet cells
  - Neuroendocrine cells
  - Intestinal epithelium for Norwalk virus production for vaccines
  - Cardiac muscle
- Tissues from the Bowhead whale

- **Propagation for Space Cell Biology**

- Cellular movement
- Signal transduction across the membrane
- Apoptosis- programmed cell death
- Gene expression
- Immunomodulation
- Recombinant gene products
- In vitro model for renal toxicity
- In vitro analysis of environmental hazards to endangered species
- Angiogenesis- formation of blood vessels
- Virus replication
- Cellular basis of space adaptation phenomena